DOI: 10.1111/all.14569

ACE2 expression and its implication in the association between COVID-19 and allergic rhinitis

To the Editor,

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), affects more than 17 million of people and results in more than 666 000 deaths all over the world. Although allergic diseases are highly prevalent globally, their risks for the development of COVID-19 remain poorly understood.

Severe acute respiratory syndrome coronavirus 2 entry host cells via angiotensin-converting enzyme II (ACE2).[1] Upregulated ACE2 expression has been associated with increased risk of COVID-19 in patients with chronic obstructive pulmonary disease, diabetes, and hypertension.[2] *ACE2* gene expression is enriched in nasal epithelial cells, highlighting the importance of nose as a portal for initial SARS-CoV-2 infection and transmission.

Allergic rhinitis (AR) is the most common disorder of nose and affects 10%-40% of the population.[3] Previous studies reported low incidences of AR in COVID-19 patients, ranging from 0% to 1.8% in China.[4,5] However, those results were generated solely based on the medical records, and AR comorbidity might not be well considered under actual emergency situation.[4,5] Moreover, the association between AR comorbidity and the disease severity of COVID-19 and the role of ACE2 in this association are largely unknown.

Here, we retrospectively analyzed 1172 etiologically confirmed COVID-19 patients discharged from Tongji Hospital, Wuhan, China from January 27, 2020 to March 10, 2020. Hospital electronic medical records were extracted and comorbidities were reevaluated by the telephone follow-up. Both multivariate logistic regression and propensity score matching (PSM) analysis were performed to exclude the influence of potential confounding variables. In addition, repository inferior turbinate tissues and cells from 29 control subjects without AR and 29 patients with AR undergoing nasal septoplasty and collected before COVID-19 era were used for the study of ACE2 expression. Human nasal epithelial cells (HNECs) were collected by epithelial scrapings of the inferior turbinates from subjects without AR. Primary HNECs were cultured with the air-liquid interface method. The cell culture was also performed before COVID-19 era, and the ACE2 expression was analyzed in this study. The RNA-seq data of nasal and bronchial brushings from 7 patients with concomitant AR and asthma and 9 healthy controls were acquired from the Gene Expression Omnibus database (GSE101720). More information regarding subjects and methods is provided in this article's Online Supplement including Tables S1-S2.

In our cohort, 115 (9.8%) patients reported physician-diagnosed AR. COVID-19 patients without AR were older than those with AR (61 [49-69] vs 54 [40 −65]; $P < .01$). Patients with AR had a higher incidence of concomitant chronic liver disease (4.4% vs 1.1%; $P = .02$), and tended to have a higher incidence of asthma comorbidity (5.2% vs 2.2%; $P = .06$) and a lower incidence of hypertension (24.4% vs 32.5%; $P = .07$) than those without AR (Table S3). After adjusting for or propensity score matching for confounding factors, including age, gender, smoking status, and comorbidities (Table S3), no significant difference in frequencies of symptoms or laboratory results was found between COVID-19 patients with and without AR (Table 1 and Table S4). Importantly, no difference in the frequencies of severe cases on admission, receiving mechanical ventilation and other treatments, or complications including severe acute respiratory syndrome was revealed for patients with and without AR either (Table 1). In PSM analysis, we were able to match 109 patients without AR to 109 patients with AR at a ratio of 1:1 (Table S3).

We next studied ACE2 expression and found its mRNA and protein expression in nasal tissues was comparable between AR patients and control subjects (Figure 1A,B). *ACE2* mRNA expression was downregulated by IL-4 and IL-13, whereas upregulated by IFN-α, IFN-γ, and TNF-α in cultured HNECs (Figure 1C). We discovered that the mRNA expression of type 2 response genes, including *ST6GAL1*, *POSTN*, and *CCL26*, was increased in nasal tissues in AR patients compared with that in non-AR controls, however, the mRNA expression of IFN response genes, including *CXCL10* and *CXCL11*, was comparable between AR patients and non-AR controls (Figure S1). *ACE2* expression positively correlated with the expression of IFN response genes, but not type 2 response genes, in nasal tissues when analyzing AR and control subjects together or separately (Figure 1D-E and Figure S2). Our findings of ACE2 expression in nasal tissues in AR patients and its relationship with the expression of IFN response genes were confirmed in nasal brushing cells from patients with concomitant AR and asthma by analyzing the public database (GSE101720) (Figures S3 and S4).

In contrast to nasal epithelial cells, *ACE2* gene expression was decreased in bronchial epithelial cells in patients with concomitant AR and allergic asthma as compared to that in healthy controls by analyzing GSE101720 datasheet (Figure S5A). *ACE2* gene expression negatively correlated with the expression of type 2 response genes *(POSTN*, *CLCA1*, and *IL1RL1)*, but not IFN response genes (*IFI6*, *CXCL10*, and *CXCL11)* in bronchial epithelial cells when analyzing all the subjects or patients with asthma and AR alone (Figure S5B and Figure S6). We further found nasal epithelial cells had higher *ACE2* and *CXCL10* expression levels, but lower *POSTN*, *CLCA1*, *CPA3*, and *IL1RL1* expression compared with those in bronchial epithelial cells (Figure S7A) in patients with concomitant AR and asthma. Consistently, the gene expression ratios of *CXCL10/ST6GAL1*, *CXCL10/POSTN*, *CXCL10/CPA3*, and *CXCL10/IL1RL1* were higher in nasal epithelial cells than in bronchial epithelial cells

Zeng and Liu joint corresponding authors

TABLE 1 Clinical characteristics, complications, and treatments of COVID-19 patients without and with AR before and after propensity score matching

Characteristics	Unmatched			Adjusted	Matched (1:1) [a]		
	Without AR	With AR	*P* value	*P* value[a]	Without AR	With AR	*P* value
Subject, N	1057	115	-	-	109	109	-
Systemic signs and symptoms, N (%)	-	-	-	-	-	-	-
Fever	823 (77.9)	98 (85.2)	.07	.09	85 (78.0)	95 (87.2)	.10
Cough	700 (66.2)	67 (58.3)	.09	.08	73 (67.0)	62 (56.9)	.16
Shortness of breath	455 (43.1)	40 (34.8)	.09	.07	52 (47.7)	38 (34.9)	.07
Fatigue	261 (24.7)	24 (20.9)	.36	.43	30 (27.5)	22 (20.2)	.27
Anorexia	253 (23.9)	21 (18.3)	.17	.09	26 (23.9)	19 (17.4)	.32
Diarrhea	182 (17.2)	24 (20.9)	.33	.42	16 (14.7)	21 (19.3)	.47
Myalgia	157 (14.9)	15 (13.0)	.60	.62	18 (16.5)	12 (11.0)	.33
Nausea and vomiting	78 (7.4)	7 (6.1)	.61	.55	5 (4.6)	5 (4.6)	.99
Headache	78 (7.4)	9 (7.8)	.86	.94	13 (11.9)	9 (8.3)	.50
Dizziness	47 (4.5)	5 (4.4)	.96	.99	3 (2.8)	5 (4.6)	.72
Severe COVID-19 cases, N (%)	183 (17.3)	16 (13.9)	.36	.79	17 (15.6)	16 (14.7)	.99
Complications, N (%)	-	-	-	-			
Acute liver injury	70 (6.6)	9 (7.8)	.56	.68	6 (5.5)	7 (6.4)	.99
ARDS	64 (6.1)	5 (4.3)	.44	.57	5 (4.6)	5 (4.6)	.99
Acute kidney injury	61 (5.8)	2 (1.7)	.08	.28	3 (2.8)	2 (1.8)	.99
Acute myocardial injury	30 (2.8)	1 (0.9)	.35	.29	3 (2.8)	1 (0.9)	.62
Shock	7 (0.7)	2 (1.7)	.21	.38	0 (0)	2 (1.8)	.50
In-hospital treatments, N (%)	-	-	-	-			
Oxygen supplementation	844 (79.9)	85 (73.9)	.14	.29	87 (79.8)	79 (72.5)	.27
Mechanical ventilation	41 (3.9)	3 (2.6)	.48	.66	4 (3.7)	3 (2.8)	.99
Antibiotic treatment	766 (72.5)	84 (73.0)	.90	.73	83 (76.1)	79 (72.5)	.64
Antiviral treatment	985 (93.2)	107 (93.0)	.95	.99	102 (93.6)	101 (92.7)	.99
Glucocorticoid therapy	273 (25.8)	22 (19.1)	.12	.06	32 (29.4)	20 (18.3)	.08
Intravenous immunoglobulin therapy	171 (16.2)	18 (15.7)	.88	.96	19 (17.4)	16 (14.7)	.71

Note: Data are presented numbers with percentages for categorical variables.

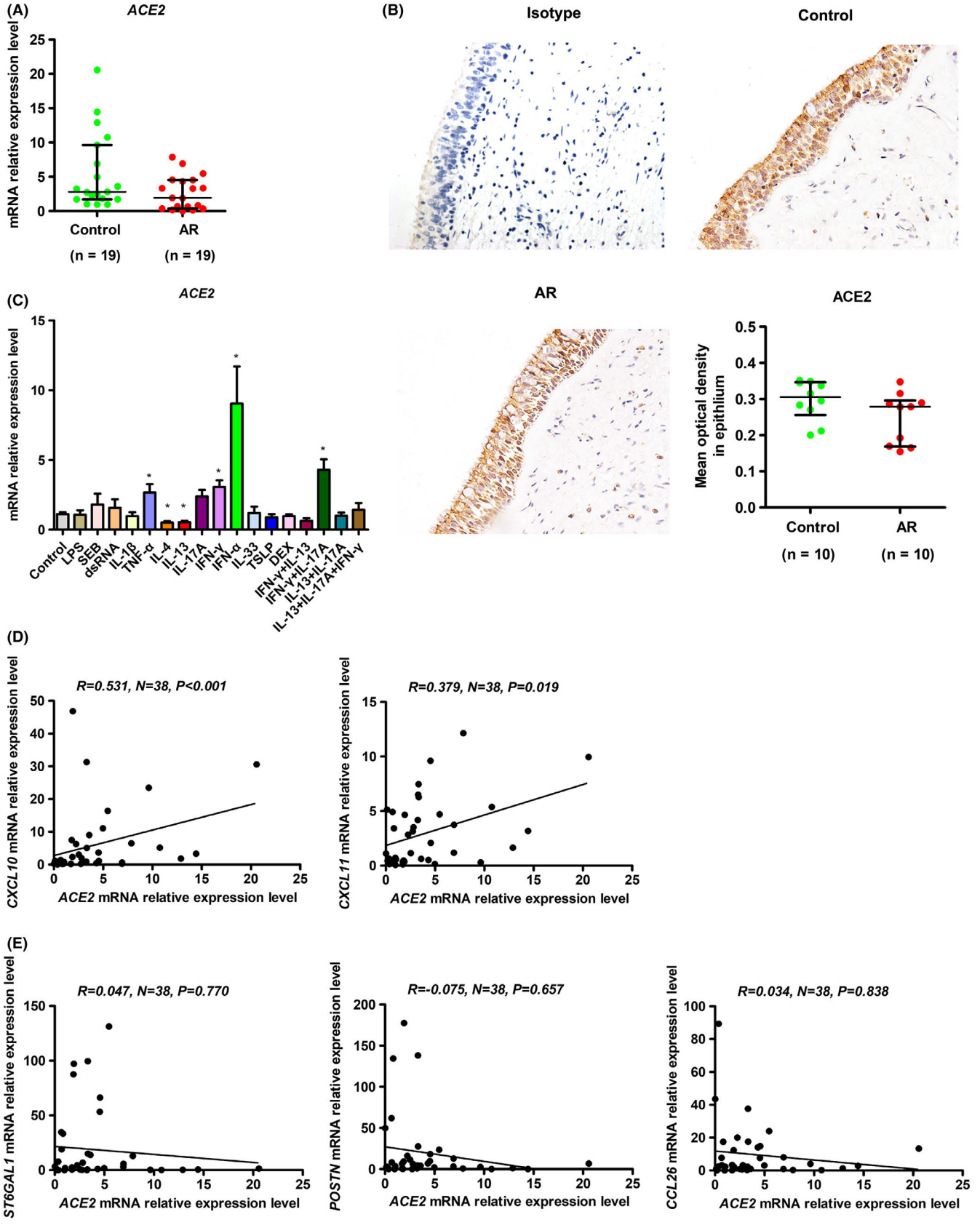
For categorical variables, Chi-square or Fisher's exact test was applied to compare the difference in proportions between groups when appropriate.

Abbreviations: AR, allergic rhinitis; ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019.

[a]Age, gender, smoking status, and all comorbidities were selected for propensity score matching. Please see Table S3.

*In adjusted logistic regression analysis, adjusted variables included age, gender, smoking, and comorbidities (hypertension, diabetes, cardiovascular disease cerebrovascular diseases, malignancy, chronic liver diseases, chronic kidney diseases, chronic rhinosinusitis, asthma, and chronic obstructive pulmonary disease).

FIGURE 1 Angiotensin-converting enzyme II expression and its correlation with IFN and type 2 response in AR patients and healthy controls. A, The mRNA expression level of *ACE2* gene in inferior turbinate tissues from AR patients and controls as detected by quantitative RT-PCR. Control, n = 19; AR, n = 19. B, Immunohistochemistry study of ACE2 expression in inferior turbinate tissues from AR patients and controls. The representative photomicrographs are shown (original magnification ×400). The staining intensity in epithelium was quantified, and the results were presented as average optical density value per unit area. Control, n = 10; AR, n = 10. C, Human nasal epithelial cells (HNECs) scraped from inferior turbinate mucosa of control subjects were cultured with an air-liquid interface method. After differentiation, HNECs were stimulated with various cytokines, dexamethasone (DEX), poly (I:C) (dsRNA), lipopolysaccharides (LPS), and staphylococcal enterotoxin B (SEB) for 6 h. After stimulation, cells were harvested for quantitative RT-PCR assay (n = 6). D and E, *ACE2* mRNA expression positively correlated with the mRNA expression of IFN response genes (*CXCL10* and *CXCL11)* (D), but not type 2 response genes (*ST6GAL1*, *POSTN*, and *CCL26* expression) (E) in tissues when analyzing AR patients and control subjects together (n = 38). Tissue data are expressed as medians and interquartile ranges and analyzed by Mann-Whitney *U* 2-tailed test. Cell culture data are expressed as medians ± SEM and analyzed by unpaired Student's *t* test. Spearman's correlation was used for correlation analysis. ACE2, angiotensin-converting enzyme II; AR, allergic rhinitis; IL, interleukin; TNF-α, tumor necrosis factor (TNF) α; IFN, interferon; TSLP, thymic stromal lymphopoietin; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; ST6GAL1, beta-galactoside alpha-2,6-sialyltransferase 1; POSTN, periostin



(Figure S7B), suggesting a predominant IFN and type 2 response in upper and lower airways, respectively, under allergic condition.

In this study, we carefully confirmed and reevaluated AR comorbidity in discharged COVID-19 patients by telephone follow-up. This may be the reason that the AR prevalence in our cohort (9.8%) was higher than those previously reported in Chinese COVID-19 patients (0%-1.8%).[4,5] The prevalence of AR in our COVID-19 cohort is comparable to that in general populations in Wuhan (9.7%).[6]

In addition, we did not find any association between AR comorbidity and disease severity in COVID-19 patients. Chhiba KD et al have recently reported that AR was not associated with an increased risk of COVID-19-related hospitalization.[7] Collectively, these results indicate that primary AR may not modify the risk for COVID-19.

In line with recent reports,[8,9] we found that type 2 cytokines downregulated, whereas IFNs upregulated *ACE2* gene expression in HNECs. However, *ACE2* expression correlated with IFN response, but not type 2 response, in nasal tissues and epithelial cells, underscoring a predominant role of IFNs in regulating *ACE2* expression in upper airways.[9]

Allergic rhinitis and allergic asthma are frequently co-occurred and may share common immune-pathogenic mechanisms such as type 2 inflammation. However, we found that patients with concomitant AR and allergic asthma had reduced *ACE2* expression in bronchial epithelial cells, which is likely regulated by type 2 response. The difference in *ACE2* expression in allergic nasal and bronchial epithelial cells is possibly related to the different tension of IFN and type 2 response in upper and lower airways under allergic condition. Therefore, the counter effect of IFN and type 2 response may have an important role in regulating ACE2 expression in airways. The reduced ACE2 expression in allergic asthmatics may suggest a lower risk for COVID-19. Low prevalence of asthma (0%-0.9%) was observed in patients with COVID-19 in several studies in China.[4] In a USA cohort, Chhiba et al[7] recorded a relatively high prevalence of asthma (14%); however, there was no significant difference in hospitalization rate or mortality between patients with and without asthma.

Our study has several limitations. First, self-reported symptoms and comorbidities might lead to the potential misestimation of the prevalence and the strength of association with the clinical outcomes. Second, we did not include fatal cases since no subsequent follow-up confirmation of comorbidities could be made for them. Third, we could not get the demographic information of subjects in the public gene dataset, and the number of subjects was limited. We therefore could not preclude the potential bias. However, the nasal epithelial cell results were consistent with our nasal tissue results derived from a relatively larger cohort.

In conclusion, for the first time, we provide the evidence that AR comorbidity may not have significant modifying effect on the development and expression of COVID-19. ACE2 expression is not altered in AR patients. *ACE2* gene expression in airways is regulated, at least in part, by the counter effect of type 2 and IFN inflammation.

KEYWORDS

allergic rhinitis, angiotensin-converting enzyme II, coronavirus disease 2019, cytokine, epithelial cell

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING INFORMATION

This study was supported by National Natural Science Foundation of China (NSFC) grants [81630024 and 81920108011 (ZL), and 81900925 (JS)], Natural Science Foundation of Hubei Province of China grant [2018CFB602 (MZ)], and National Key R&D Program of China (2018YFC0116800).

Hai Wang[1]
Jia Song[1]
Yin Yao[1]
Yi-Ke Deng[1]
Zhi-Chao Wang[1]
Bo Liao[1]
Jin Ma[1]
Chao He[1]
Li Pan[1]
Yang Liu[1]
Jun-Gang Xie[2,3]
Ming Zeng[1]
Zheng Liu[1,3]

[1]*Department of Otolaryngology-Head and Neck Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*
[2]*Department of Respiratory and Critical Care Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*
[3]*Key Laboratory of Respiratory Diseases of Ministry of Health, Wuhan, China*

Correspondence
Zheng Liu and Ming Zeng, Department of Otolaryngology-Head and Neck Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095 Jiefang Avenue, Wuhan 430030, China.
Emails: zhengliuent@hotmail.com (ZL); zmsx77@163.com
Wang, Song and Yao contributed equally

ORCID

Jun-Gang Xie https://orcid.org/0000-0001-9037-3027
Zheng Liu https://orcid.org/0000-0002-4168-6702

REFERENCES

1. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol*. 2020;5(4):562-569.
2. Li Y, Zhou W, Yang L, You R. Physiological and pathological regulation of ACE2, the SARS-CoV-2 receptor. *Pharmacol Res*. 2020;157:104833.
3. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action. *World Allergy Organ J*. 2014;7(1):12.
4. Zhang JJ, Dong X, Cao YY, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy*. 2020;75(7):1730-1741.
5. Jian L, Yi W, Zhang N, et al. Perspective: COVID-19, implications of nasal diseases and consequences for their management. *J Allergy Clin Immunol*. 2020;146(1):67-69.
6. Wang XD, Zheng M, Lou HF, et al. An increased prevalence of self-reported allergic rhinitis in major Chinese cities from 2005 to 2011. *Allergy*. 2016;71(8):1170-1180.

7. Chhiba KD, Patel GB, Vu THT, et al. Prevalence and characterization of asthma in hospitalized and non-hospitalized patients with COVID-19. *J Allergy Clin Immunol*. 2020;146(2):307.
8. Kimura H, Francisco D, Conway M, et al. Type 2 inflammation modulates ACE2 and TMPRSS2 in airway epithelial cells. *J Allergy Clin Immunol*. 2020;146(1):80.
9. Ziegler CGK, Allon SJ, Nyquist SK, et al. SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. *Cell*. 2020;181(5):1016.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

DOI: 10.1111/all.14584

Thirty-six COVID-19 cases preventively vaccinated with mumps-measles-rubella vaccine: All mild course

To the Editor,

We would like to report here on our clinical observations in 255 subjects, vaccinated in our Center since the start of the Coronavirus disease-2019 (COVID-19) pandemic, with the mumps-measles-rubella (MMR) vaccine and of whom thirty-six have presented COVID-19, all with a remarkably mild course.

In the light of the COVID-19 pandemic, observing the highly contagious and virulent nature of the virus, new to mankind and for which no actual treatment nor vaccination exists, we have been searching for methods to enhance innate immunity. Moreover, the pandemic started in our country just after a rise in measles cases had motivated the Ministry of Health to recommend measles re-vaccination. Aware of the existence of trained immunity, we decided to apply this concept March 2020 onward recommending MMR vaccination, especially among family members of COVID-19 cases. In June 2020, the American Society for Microbiology (AMS) speculated in a press release that "the MMR vaccine could serve as a preventive measure to dampen …. COVID-19 infection."

In a prospective observational trial, we followed MMR vaccinated subjects searching for COVID-19 cases (ethics committee: CONBIOÉTICA-09-CEI-018-20160729). All patients were vaccinated subcutaneously with 0.5 mL of the MMR vaccine containing live-attenuated virus (≥1000 $CCID_{50}$ of measles, ≥5000 $CCID_{50}$ of mumps and ≥1000 $CCID_{50}$ of Rubella virus) and follow-up was given by (bi)monthly phone calls or contact via electronic media. COVID-19 infection was considered confirmed with a positive result of the SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR), the detection of SARS-CoV-2 specific antibodies or the combined presence of a direct contact with a confirmed case plus anosmia/ageusia plus at least two classic symptoms. Direct contact with a confirmed case, accompanied by classic symptoms, but without olfactory nor gustatory alterations were considered highly probable cases. We graded the clinical severity of COVID-19 on a simplified scale we considered more suitable in an out-patient setting, see Table 1.

Among the 255 vaccinated subjects, there are 24 confirmed and 12 (highly) probable COVID-19 cases, thirteen of them with hypertension, diabetes, obesity, smoker, or uncontrolled asthma as possible risk-factors. As people are generally very reluctant to go to a laboratory or take a chest X-ray, we have installed close follow-up in probable positive cases with pulse oximetry and home peak expiratory flow rate (PEF) measurements. All received general supportive measures and the policy toward fever was permissive, keeping paracetamol use to a minimum. Some received off-label high-dose ivermectin the first 2 days. All had minor respiratory symptoms at most; only one uncontrolled asthmatic had 1 day hypoxemia. None presented respiratory insufficiency to the degree of needing oxygen.

The concept of trained immunity based on a heterologous immune response with nonspecific memory dates back about a decade ago and refers to the enhanced immune response to a certain pathogen, after being exposed (by vaccination or natural illness) to another nonrelated pathogen[1] and Matricardi analyzed this in the context of the COVID-pandemic.[2] The immune reaction after a subsequent exposure to a nonrelated pathogen is faster in onset and accompanied by an increased production of certain cytokines. As such, trained immunity by nature is nonspecific and carried by cells from the innate branch of the immune system, especially monocytes and NK-cells. Thus, it seems the innate immune system also has a certain kind of memory, as this enhanced response to a second pathogen can still clearly be detected 3 months, and in a lesser degree up to 12 months later. Interestingly, heterologous T helper-cell (Th)1 and Th17 adaptive immune responses to nonrelated pathogens also remained intensely elevated, even 1 year later.[3]

At molecular level a rise in aerobic glycolysis, oxidative phosphorylation and glutamine metabolism have been described in monocytes, induced by exposure to BCG, β-1,3-(D)-glucan from *Candida albicans* or heat-killed bacteria, that is, via pathways

Abbreviations: BCG, Bacillus Calmette-Guérin; COVID-19, Coronavirus disease-2019; MMR, mumps-measles-rubella; NOD, Nucleotide-binding oligomerization domain-containing protein; RT-PCR, reverse transcription-polymerase chain reaction; Th, T helper-cell; TLR, Toll-like receptor; TNF-α, Tumor necrosis factor-alpha.